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Correlation of Retention of Tumor Methylmercaptopurine Riboside-5′-Phosphate with Effectiveness in CD8F1 Murine Mammary Tumor Regression

L. Dee Nord, Robert L. Stolfi, Joseph R. Colofiore and Daniel S. Martin*

DEPARTMENT OF CANCER RESEARCH, CATHOLIC MEDICAL CENTER, WOODHAVEN, NY 11421; AND MEMORIAL SLOAN-KETTERING CANCER CENTER, NEW YORK, NY 10021, U.S.A.

ABSTRACT. Treatment with a combination (PMA) of (*N*-phosphonacetyl)-L-aspartic acid (PALA), methylmercaptopurine riboside (MMPR), and 6-aminonicotinamide (6AN) induced partial regressions of CD8F1 murine mammary tumors and provided for tumor growth inhibition without regression of Colon 38 tumors. HPLC-nucleotide pool analysis of CD8 mammary tumors obtained at various times after treatment with PMA revealed that MMPR-5′-phosphate, which inhibits *de novo* purine nucleotide biosynthesis, was constant at levels of approximately 2.5 nmol/mg protein for 72 hr after treatment. In contrast, the MMPR-5′-phosphate levels of C38 tumors decreased from 24-hr levels at 1.5 nmol/mg protein with a half-time of about 24 hr. Treatment of CD8 tumor-bearing mice with iodotubercidin, a potent inhibitor of adenosine/MMPR kinase, at various times after PMA, reversed both the accumulation of high levels of MMPR-5′-phosphate and the number of partial tumor regressions. These data demonstrate that a cycle of MMPR rephosphorylation is active in the CD8 mammary tumor and suggest that this recycling of MMPR is important for the optimal effect of PMA treatment. BIOCHEM PHARMACOL 51;5:621–627, 1996.

KEY WORDS. methylmercaptopurine riboside; *N*-(phosphonacetyl)-L-aspartic acid; 6-aminonicotinamide; mammary carcinoma; tumor regression; iodotubercidin

A number of investigators have observed a cascade of events induced by treatment of cancer cells with DNA-damaging agents [1–6]. According to this scheme, many anticancer agents can induce a high degree of DNA breaks. DNA strand breaks then serve to activate the nuclear enzyme poly(ADP-ribose)polymerase, which functions to inactivate certain proliferation-related proteins by attaching polymers of ADP-ribose at distinct sites on the protein at the expense of the substrate NAD. If sufficient NAD is utilized, the shuttling of reducing equivalents is impaired leading to inefficient regeneration of ADP to ATP and eventually to ATP depletion and subsequent cell death. Based on the view that DNA damage leading to cell death may be related to cellular ATP levels [7], a drug combination consisting of PALA†, MMPR and 6AN, otherwise referred to by the acronym PMA, was developed with the primary purpose of depleting ATP levels. We have since reported that the PMA combination is effective in inducing the regression of CD8F1 mammary tumors *in vivo* and in inducing depletion of ATP levels as tumors are undergoing regression [8]. Moreover, combination treatment with PMA has been shown to improve the efficacy of other cancer chemotherapeutic agents [9], including 5-fluorouracil [8], Adriamycin® [10], 5-fluorouracil + Adriamycin [11], and phenylalanine mustard [9]; and also another treatment modality (radiation) [12].

Although all three PMA components are necessary in combination to induce tumor regressions, the primary effector of ATP depletion is MMPR [7]. MMPR is metabolized to the corresponding nucleoside 5′-phosphate by the action of adenosine kinase. Although small amounts of the higher phosphate derivatives of MMPR have been detected under certain conditions of extended drug treatment [13], the major metabolite in mammalian cells is the 5′-monophosphate derivative, MMPR-5P, which is a potent inhibitor of the first enzyme of *de novo* purine biosynthesis, amidophosphoribosyltransferase [14, 15].

Since PMA treatment of another solid tumor, Colon 38, resulted in tumor growth inhibition without regression, we evaluated MMPR metabolism in CD8 and C38 tumors after PMA treatment. It was found that in the sensitive CD8F1 tumors, MMPR-5P was retained at a high level over 72 hr even as tumors were undergoing regression. In the less sensi-

* Corresponding author: Dr. L. Dee Nord, Catholic Medical Center, Department of Cancer Research, 89-15 Woodhaven Boulevard, Woodhaven, NY 11421. Tel. (718) 441-1147; FAX (718) 846-6885.

† *Abbreviations:* PALA, *N*-(phosphonacetyl)-L-aspartic acid; MMPR, methylmercaptopurine riboside; MMPR-5P, the 5′-phosphate derivative of MMPR; 6AN, 6-aminonicotinamide; PMA, an acronym for the triple chemotherapeutic combination of PALA, MMPR, and 6AN; PR, partial regression; IodoT, iodotubercidin; 6ANAD, the 6-AN analog of NAD; 6ANADP, the 6AN analog of NADP; and 6PG, 6-phosphogluconate.

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tive C38 tumors, PMA provided only tumor growth inhibition, and MMPR-5P was not retained but was lost with a half-time of 24 hr.

The relevance of MMPR-5P retention to tumor regression was further investigated with the use of IodoT, an inhibitor of adenosine kinase [16]. When given 6 or 24 hr after PMA, to allow for an initial activation of MMPR, IodoT prevented the extended accumulation of MMPR-5P and reversed the chemotherapeutic effect of PMA. These results indicate the importance of an extended high level of intracellular MMPR-5P for the optimal chemotherapeutic effect of PMA treatment.

MATERIALS AND METHODS

MMPR and 6AN were purchased from the Sigma Chemical Co. Iodotubercidin was purchased from Research Biochemicals, Inc. PALA was provided by the NCI. Mice used in Colon 38 experiments were purchased from Taconic Farms.

Tumor Models

CD8F1 SPONTANEOUS MAMMARY TUMOR. The CD8F1 spontaneous mammary tumor model has been described previously [17, 18] and was included in the murine testing panel of the National Cancer Drug Screening Program [19]. CD8F1 hybrid mice bearing single spontaneous, autochthonous breast tumors were selected from the mouse colony. A tumor brei was made from 3–4 of these tumors and transplanted subcutaneously in 3-month-old syngeneic Balb/C × DBA/8 (CD8F1) mice. In 3–4 weeks, tumor-bearing mice were distributed among treatment groups, and experiments were conducted in these first passage transplants. The average tumor weights were around 150 mg at the beginning of treatment.

As in all spontaneous tumors, whether human or murine, each individual cancer has a heterogeneous neoplastic cell population. Since each experiment consisted of a brei composed of several different spontaneous tumors, the neoplastic cell composition was somewhat different from experiment to experiment, resulting in certain quantitative differences between experiments. However, each experiment had its own control, and the results are quantitatively relevant within individual experiments, as are trends among experiments.

COLON 38 TUMORS. Colon 38 tumors were carried in C57BL mice, and experiments were performed in either C57BL (Expt. 2) or BDF1 (B6 × D2) mice (Expts. 1 and 3).

PMA Drug Regimen

The standard PMA regimen consisted of $PALA_{100}$ followed after 17 hr with $MMPR_{150}$ and $6AN_{10}$, where subscripts refer to the dose in mg/kg. IodoT at 3 mg/kg was administered at 6 or 24 hr after MMPR + 6AN treatment. All drugs were administered by i.p. injection.

HPLC Methodology

Mice were anesthetized i.p. with pentobarbital. Tumors were excised and immediately freeze-clamped using a pair of tongs cooled in liquid nitrogen. Frozen tumors were homogenized in ice-cold 0.4 N perchloric acid (1 mL/0.1 g tumor weight). The acid-insoluble fraction was removed by centrifugation (10,000 g for 5 min), and the acid-soluble fraction was neutralized by extraction with a mixture of tri-*n*-octylamine in Freon [20] and analyzed by HPLC. MMRP-SP values were normalized to the protein content of the acid-insoluble fraction, and the values are expressed as nanomoles per milligram protein. Protein was solubilized in 0.5 N NaOH and quantitated [21]. HPLC analysis was performed using a Waters 840 HPLC, a WISP autosampler, and tandem UV detection at 254 and 290 nm. Nucleotides were separated on a Whatman SAX column starting with 3 mM ammonium phosphate, pH 3.1, and proceeding in two steps to 70% of high salt buffer, 0.5 M ammonium phosphate, pH 5.4. The injection volume of each sample was 200 μL. The run time was 70 min at a flow rate of 1.5 mL/min with a 20-min equilibration period between analyses.

RESULTS

Effect of PMA on Tumor Growth

The effect of PMA treatment on advanced CD8F1 tumor growth is shown in Fig. 1. Although the effect of PMA treatment may vary somewhat from experiment to experiment, treatment with PMA provided a consistent number of tumor regressions. The data in Fig. 1 are representative of the CD8F1 tumor-regressing effect of a single course of PMA treatment. As shown, there was an immediate arrest of tumor growth at the point of treatment with $MMPR_{150}$ + $6AN_{10}$ (17 hr following $PALA_{100}$ treatment), leading to a decrease in tumor

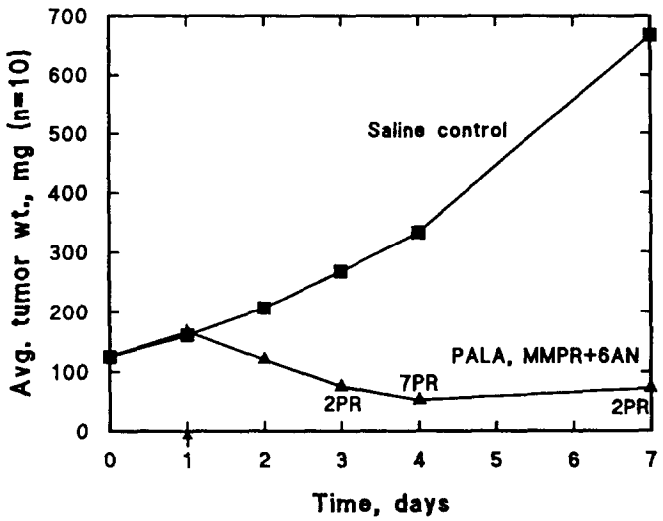

FIG. 1. Effect of the first course of PMA treatment on first-passage CD8F1 spontaneous-mammary tumor growth. CD8F1 mammary tumor-bearing mice were treated with saline (■) or with the PMA combination (▲): $PALA_{100}$, given at 17 hr prior to the administration on day 1 (↑) of simultaneous $MMPR_{150}$ and $6AN_{10}$. Subscripts refer to the dose in mg/kg. The number of partial tumor regressions (PR) per group of ten mice is shown. Tumors averaged 125 mg at the initiation of chemotherapy.

mass as indicated by the mean tumor weight and the number of PRs at each time point. In this particular experiment, the time of maximal effect (7 PR among 10 tumors) was observed 3 days following MMPR + 6AN treatment.

In contrast, treatment of Colon 38 tumor-bearing mice with PMA provided only tumor growth inhibition, as shown by the representative experiment in Fig. 2. At both time points, the tumors of the PMA-treated mice were significantly smaller than those of the saline control, but there were no tumor regressions.

Comparative Levels of MMPR-5P in CD8F1 and Colon 38 Tumors

The levels of MMPR-5P from CD8F1 tumors were compared with the levels obtained in C38 tumors (Table 1). In a series of experiments in CD8F1 tumors, the MMPR-5P levels remained nearly constant over 72 hr following treatment with PMA. However, the levels of MMPR-5P in C38 tumors were reduced over time with a half-life of 24 hr. This extended retention of MMPR-5P was found in each of the six experiments with CD8F1 tumors. The absolute level of MMPR-5P varied among experiments, but the trend of MMPR-5P retention over time was consistent.

Effect on PMA-Induced Tumor Regression by Prevention of MMPR-5P Accumulation with IodoT, an Inhibitor of Adenosine Kinase

The contribution of prolonged tumor MMPR-5P levels to the efficacy of PMA treatment of CD8F1 tumors was examined by inhibiting the synthesis of MMPR-5P by means of IodoT administration. As shown in a representative chemotherapy experiment (Table 2), PMA-induced regressions of advanced CD8F1 tumor, averaging 254 mg at the initiation of treatment, were reversed from 9 PR to 2 PR by the addition of IodoT, and tumor weight increased significantly from a mean of 66 to 206 mg. IodoT was given 6 hr after MMPR + 6AN to allow an initial activation of MMPR by adenosine kinase. In a similar experiment, IodoT reversed PMA-induced tumor regressions when given 6 or 24 hr after MMPR + 6AN (Fig. 3). For example, on day 4 there were 5/10 regressions in the PMA group, but only 2/10 in the IodoT 24-hr group and 0/10 in the IodoT 6-hr group. MMPR-5P levels were reduced from control values of 3.0 to ≤0.2 nmol/mg (Table 3) in both IodoT-treated groups. The data from Table 3 and Fig. 3 were generated using mice bearing CD8F1 tumors that originated from the same tumor brei. IodoT given 6 hr after MMPR was sufficient to prevent the accumulation of MMPR-5P, and little MMPR-5P was found in CD8F1 tumors 18 hr later. In addition, an incremental degree of reversal was observed depending on the time (6 or 24 hr) of IodoT administration following MMPR + 6AN (Fig. 3). The superior chemotherapeutic effect of the PMA combination, therefore, appeared to correlate with longer exposure to MMPR-5P.

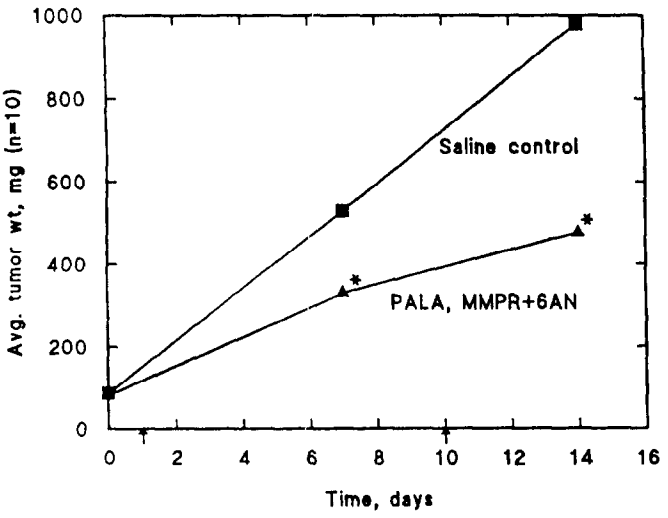

FIG. 2. Effect of PMA treatment on C38 colon tumor growth. Groups of ten C38 colon tumor-bearing mice were treated with saline or the PMA regimen for two courses of treatment. MMPR and 6AN were administered (↑) simultaneously 17 hr after PALA. Key: (*) significantly different ($P < 0.05$) compared with the saline control. Tumors averaged 80 mg at the onset of chemotherapy.

DISCUSSION

The combination of PALA, MMPR, and 6AN (PMA) was designed to modulate cellular energy [7]. Although there are likely numerous metabolic consequences produced by treatment with these drugs, from the viewpoint of metabolic energy, the PMA combination may be inhibiting cellular energy by (1) decreasing the *availability* of ATP owing to restriction of *de novo* purine synthesis by treatment with MMPR, and (2) preventing *regeneration* of ADP to ATP by treatment with 6AN.

MMPR is activated mainly to the 5′-phosphate derivative, MMPR-5P, by adenosine kinase [13]. The primary action of MMPR-5P is then to restrict the *availability* of ATP by inhibiting the first enzyme of *de novo* purine biosynthesis, amidophosphoribosyl transferase [22].

ATP *regeneration* occurs through substrate-level phosphorylation at the level of phosphoglycerate kinase in the glycolytic pathway or by oxidative phosphorylation (see Ref. 23 for a review) in the mitochondria. Both processes are NADH dependent and are subject to complex metabolic regulation. The relative contributions to ATP regeneration vary among different tissues, but conditions associated with solid tumors (i.e. hypoxia) have been thought to favor glycolysis as a preferred contributor to ATP regeneration [24, 25]. Treatment with PMA may inhibit ATP regeneration by either, or perhaps both, of these pathways [26]. In this regard, 6AN is a substrate for nicotinamide-metabolizing enzymes [27] and is metabolized first to the corresponding nucleotide monophosphate and further to analogues of NAD and NADP. At the 6ANAD(P) level, these metabolites function to inhibit NAD(P)-dependent reactions. For example, 6PG dehydrogenase, the second enzyme of the oxidative branch of the pentose phosphate pathway [28], is inhibited following 6AN treatment. In

TABLE 1. Comparative levels of MMPR-5P in CD8 and C38 tumors following treatment with PMA

Tumors, Expt. No.	MMPR-5P levels (nmol/mg protein) at time after treatment				
	8 hr	24 hr	48 hr	72 hr	96 hr
CD8 mammary					
1	—*	1.5 ± 0.6	1.2 ± 0.5	—	—
2	—	4.3 ± 2.1	1.9 ± 0.6	2.9 ± 0.7	—
3	—	3.2 ± 1.2	3.0 ± 0.5	2.9 ± 1.2	—
4	—	3.2 ± 1.3	3.0 ± 0.8	1.5 ± 0.8	1.5 ± 0.3
5	—	2.6 ± 0.9	2.6 ± 1.0	—	—
6	—	2.3 ± 1.1	3.0 ± 1.6	—	—
Mean		2.9	2.5	2.4	
C38 colon					
1	—	1.3 ± 0.3	0.8 ± 0.3	0.4 ± 0.1	—
2	—	1.3 ± 0.9	0.9 ± 0.6	0.2 ± 0.1	0.2 ± 0.1
3	2.5 ± 0.8	1.9 ± 0.6	0.8 ± 0.2	0.4 ± 0.1	—
Mean		1.5	0.8	0.3	

Mice bearing CD8F1 mammary or Colon 38 tumors were treated with the combination of $PALA_{100}$, $MMPR_{150}$ and $6AN_{10}$ (PMA). Subscripts denote the delivered dose in mg/kg. Mice were treated with PALA followed 17 hr later by the simultaneous delivery of MMPR + 6AN. Each treatment group consisted of from 6 to 10 mice. Values are means ± SD.

* No cohort for indicated time point and, therefore, no determination of the MMPR-5P level.

CD8F1-tumor bearing mice, inhibition of 6PG dehydrogenase following 6AN treatment causes a large (100-fold) increase in the levels of 6PG [8]. There are likely other more subtle inhibitory actions attributable to 6ANAD or 6ANADP, but the accumulation of 6PG has been used as a functional indicator of 6AN metabolite formation and is physiologically significant since 6PG inhibits glycolysis by a feedback inhibition of phosphoglucose isomerase [29]. Thus, the accumulation of 6PG may inhibit ATP regeneration by inhibiting the flux through the glycolytic pathway, and the oxidative phosphorylation pathway for ATP regeneration may be impaired by inhibition of an NAD(P)-dependent enzyme with 6ANAD(P).

The observation that ATP levels are reduced following PMA treatment has been well documented [8–10], and using PMA treatment as a staging strategy to deplete ATP offers the possibility of further ATP depletion and tumor regression by, for example, addition of DNA-damaging agents, which are thought to further reduce ATP levels by a poly(ADP-ribose)-polymerase-dependent mechanism. In this regard it is of importance to identify which determinants of ATP homeostasis are impaired by PMA treatment to design more effective strategies.

In the present study, the levels of MMPR-5P were investigated in the sensitive CD8 mammary tumor and the less sensitive Colon 38 tumor to determine the relative importance of inhibition of *de novo* purine biosynthesis to the efficacy of PMA treatment. We show here that a prolonged inhibition of the *de novo* purine pathway as suggested by the retention of MMPR-5′-phosphate correlates with superior chemotherapeutic benefit and a higher incidence of regression. This was confirmed further by the reversal of the antitumor effect by IodoT, which inhibited the synthesis of MMPR-5P. In addition, better chemotherapeutic activity was found with longer exposure to MMPR-5P, since treatment with IodoT at 24 hr after MMPR + 6AN was superior to IodoT at 6 hr after MMPR + 6AN.

These data confirm that maintenance of MMPR-5P is a dynamic process requiring ATP-dependent resynthesis from MMPR [14]. When adenosine kinase is blocked, MMPR-5P levels are severely depleted within 18 hr. Similar results were

TABLE 2. Reversal of PMA-induced chemotherapeutic effect by IodoT in CD8F1 tumors over one course of treatment*

Treatment†	Dead/total	Tumor weight (mg)	PR‡/total
(1) $PALA_{100}$ —17 hr → $MMPR_{150}$ + $6AN_{10}$	0/10	66	9/10
(2) $PALA_{100}$ —17 hr → $MMPR_{150}$ + $6AN_{10}$ —6 hr → $IodoT_3$	0/10	206§	2/10

The adenosine kinase inhibitor IodoT was used to prevent the synthesis of MMPR-5P following PMA (PALA, MMPR + 6AN) treatment, and the resulting effect on tumor growth and regression was observed.

* Exp. 2775M: First passage CD8F1 tumors averaging 254 mg at the time of initiation of treatment.

† Observations were recorded 1 week after initiation of the indicated treatment. Subscripts refer to doses in mg/kg.

‡ PR = tumor regression of 50% or more compared with the pretreatment tumor weight.

§ Significantly different when compared with the value from group 1 (Student's *t*-test; $P < 0.05$).

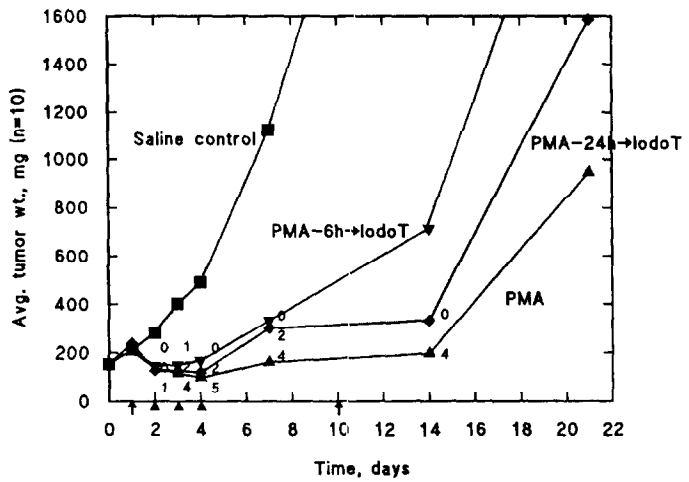

FIG. 3. Effect of IodoT on PMA-induced tumor regression when administered 6 or 24 hr after MMPR. Groups of ten CD8F1 tumor-bearing mice were treated with saline, PMA, or with PMA followed by IodoT (3 mg/kg). IodoT was given either 6 or 24 hr after MMPR + 6AN (↑) treatment. The number of partial tumor regressions (PR) are recorded next to the symbol for each group. Tumors were harvested at the time indicated (▲) along the baseline for determination of MMPR-5′-phosphate levels (see Table 3). Tumors averaged 154 mg at the initiation of chemotherapy.

obtained *in vitro* when mouse lymphoma cells were incubated with various concentrations of MMPR and then withdrawn to fresh growth medium containing no MMPR; at each concentration of MMPR in the medium a steady-state concentration of MMPR-5P was attained, and each level was reduced by 90% over the first hour of incubation in a drug-free medium [14]. In CD8F1 tumors, it is not known whether the MMPR is recycled within the cell or whether there is a resupply of MMPR from a "depot" source such as erythrocytes. However, since the initial synthesis of MMPR-5P in C38 (2.5 nmol/mg at 8 hr after MMPR + 6AN) was similar to that observed in CD8F1 tumors but was not retained, it would appear that MMPR to a large extent must be recycled within the CD8 tumor. These data also suggest that in the CD8F1 tumors, the rate of MMPR phosphorylation is high compared with the rate of dephosphorylation.

Since the pH optimum of adenosine kinase for MMPR is at a lower pH of 5.5 [30], any intracellular acidification such as may be found in a hypoxic environment would seem to favor an increased synthesis of MMPR-5P. The range of pH in CD8F1 tumors is not known presently, but acid pH values approaching 5.5 have been shown to occur under certain therapeutic conditions in experimental tumors [25]. In addition, a drop in cellular pH by as much as 1 unit has been associated with cells undergoing apoptosis [31, 32]. While the status of such cells for uptake of nucleosides is unknown, apoptotic cells maintain integrity of the plasma membrane and are eventually engulfed by neighboring cells. Thus, whether or not apoptotic cells are capable of actually concentrating a higher steady-state level of MMPR-5P than non-apoptotic cells, this represents a potential mechanism for MMPR delivery, and may apply to other "recyclable" drugs as well.

The reversal of MMPR-induced cytotoxicity by IodoT and certain analogs has been used *in vitro* to indicate adenosine kinase inhibition in cultured CEM cells [33]. The reversal of PMA activity by IodoT in this study is less than might be expected, since only 6-hr exposure to PMA prior to IodoT treatment still produced good tumor growth inhibition. However, IodoT prevents the salvage of adenosine for restoration of the adenylate pools and also leads to local increases in vasoactive adenosine resulting in antiinflammatory activity [34, 35]. It has also been suggested that IodoT may act as a general protein kinase inhibitor [36].

One further effect of MMPR is the elevation of 5-phospho-α-D-ribosyl pyrophosphate, which is required for anabolism of 6AN. This modulation of 6AN activation by MMPR may well be complete prior to the addition of IodoT in these experiments, and the antitumor effects of PALA and 6AN may be affected minimally by IodoT treatment.

It is also of interest that the phosphorylation of MMPR is not impaired even as the tumors are undergoing regression, since ATP levels are reduced by PMA treatment [8]. While severe depletion of ATP levels is thought to be incompatible with cell survival, there appears to be a hierarchy of ATP utilization within the cell. In general, the K_m for ATP of many regulatory ATP-requiring enzymes is in the low micromolar range, whereas normal intracellular ATP levels are in the millimolar range. The K_m for ATP of adenosine kinase is on the order of 2 μM [37, 38]. Even in the case of severe (80%) ATP

TABLE 3. Effect of IodoT, an adenosine kinase inhibitor, on MMPR-5P levels when administered 6 or 24 hr after MMPR

	MMPR-5P levels (nmol/mg protein) at time after MMPR + 6AN		
Treatment	**24 hr**	**48 hr**	**72 hr**
(1) PMA	3.2 ± 1.2	3.0 ± 0.5	2.9 ± 1.2
(2) PMA → 6 hr → $IodoT_3$	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.5
(3) PMA → 24 hr → $IodoT_3$	ND*	0.2 ± 0.1	0.5 ± 0.3

Groups of eight CD8F1 tumor-bearing mice were treated with the PMA combination ($PALA_{100}$, $MMPR_{150}$ and $6AN_{10}$). Subscripts refer to dose in mg/kg. IodoT (3 mg/kg) was given 6 or 24 hr after MMPR + 6AN treatment (PALA given 17 hr earlier) to allow an initial activation of MMPR to the 5′-phosphate derivative. Values are means ± SD.

* ND = not determined.

depletion, there would seem to be sufficient ATP to maintain the function of the most critical (high affinity-low V_{max}) reactions, such as maintaining the phosphorylation status of critical regulatory proteins. However, as far as bulk ATP utilization, plasma membrane Na^+/K^+ ATPase and protein biosynthesis stand out as the primary ATP consumers [23] and, in general, all known animal cation transport ATPase proteins function in a low affinity-high V_{max} (K_m for ATP of 0.4 to 2 mM) mode and are more sensitive to inhibition by reduced ATP levels [39]. These processes may initially be more susceptible to PMA-induced ATP depletion, and PMA treatment may exert tumor cytotoxic effects by microregional or compartmental depletion of ATP similar to the effects described for tumor-bearing rats treated with hyperglycemia/hyperthermia [25], while other regional or compartmentalized ATP-dependent processes are less affected.

Further, while ATP depletion in the CD8F1 tumor precedes the depletion of the other nucleoside triphosphates, it is possible that the concomitant lowering of other nucleoside triphosphates such as GTP may be more critical for certain aspects of cell growth [40, 41].

In conclusion, in the process of dissecting certain biochemical determinants of PMA action, we have examined the metabolism of MMPR to the active metabolite MMPR-5P in two tumor types. In the sensitive CD8 tumors, MMPR-5P was retained at a high "steady-state" level over 72 hr. In the less sensitive Colon 38 tumors, MMPR was initially (within 8 hr) metabolized to levels comparable to those attained in the sensitive tumors, but was lost from the tumors with a half-life of 24 hr. When the synthesis of MMPR-5P was inhibited by administration of IodoT to PMA-treated CD8-tumor bearing mice, MMPR-5P levels were severely depleted and the antitumor effect of PMA was reversed, as determined by both reversal of tumor growth inhibition and a decrease in the number of tumor regressions. These data indicate that in the context of PMA treatment, persistent high levels of MMPR-5P contribute to optimal chemotherapeutic efficacy.

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